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Xerocomus porophyllus sp. nov., morphologically intermediate between *Phylloporus* and *Xerocomus*

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Abstract— *Xerocomus porophyllus*, discovered from Dinghushan Biosphere Reserve in Guangdong province (southern China), is described as a new species. It is a morphologically intermediate taxon between *Phylloporus* and *Xerocomus* since its hymenophore is lamellate around the stipe but strongly anastomosing to distinctly faveolate or poroid towards pileus margin. Molecular analyses of nuclear rDNA ITS and LSU sequences, however, indicate that it has a closer genetic relationship with *Xerocomus* than with *Phylloporus*.

Key words— *Basidiomycota*, *Boletaceae*, *Boletales*, phylogeny, taxonomy

Introduction

Phylloporus Quél. and *Xerocomus* Quél. belong to the family *Boletaceae* and have a close relationship, whether *Phylloporus* has been considered as a primitive group in the family (Corner 1972) or as a derived genus next to *Xerocomus* (Binder 1999; Binder & Hibbett 2006). Molecular data have shown the *Xerocomus subtomentosus* complex to be closely related to *Phylloporus* (Binder 1999). The lamellate hymenophore in *Phylloporus* and the wide poroid or tubular one in *Xerocomus* are still used as the main morphological characters for differentiating the two genera (Neves & Halling 2010; Neves et al. 2012).

Worldwide, *Phylloporus* encompasses about 70 named species (Neves et al. 2012), 21 of which have been reported from China (Teng 1963; Zang & Zeng 1978; Li et al. 1992; Bi et al. 1993, 1997; Dai & Li 1994; Zang et al. 1996; Zeng et al. 2012). However, two Chinese records should be excluded because the type specimen of *Phylloporus scabrosu*s M. Zang is a *Lentinus* sp., and Chinese exsiccatae labelled as "*Phylloporus rhodoxanthus*" were misidentified (Zeng et al. 2012).

Although some mycologists have incorporated *Xerocomus* into *Boletus* (Corner 1972, Kirk et al. 2008), other authors still treat it as an independent

genus (Watling & Hills 2005, Hills 2008, Neves et al. 2012, Zeng et al. 2012). Until now, more than 210 species and varieties of *Xerocomus* have been registered (www.indexfungorum.org), of which at least 46 species have been reported from China (Zhang et al. 2012).

Recently, an interesting bolete was recorded in Dinghushan Biosphere Reserve in Guangdong province (southern China) and found to differ from all known taxa. Although its hymenophore is consistently lamellate (*Phylloporus*-like) around the stipe, it is also obviously faveolate or tubular (*Xerocomus*-like) towards the pileal margin. Molecular analyses of nuclear rDNA ITS and LSU sequences, however, indicated it to have a closer genetic relationship with *Xerocomus* than with *Phylloporus*. Further investigation revealed that it is a new species to science, and therefore it is formally described here. The holotype is deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM).

Materials & methods

Specimens were annotated and photographed in the field, and dried in an electric drier. Color descriptions were made according to Kornerup & Wanscher (1978). Tissue sections were mounted in 5% KOH or 1% Congo Red for microscopic examination. Dimensions of basidiospores are given using the notation (a–)b–c(–d), where 'b–c' represents 90% or more of the measured values of at least 30 basidiospores, and the parenthetical 'a' and 'd' are the extreme values. Lm refers to the average length of all basidiospores standard deviation; Wm refers to the average width of all basidiospores standard deviation; Qm refers to the average Q of all basidiospores standard deviation; and n refers to the number of spores measured per specimen. Basidiospores were also observed under a scanning electron microscope (SEM). Light micrographs were taken using an Olympus BX51 trinocular phase contrast microscope and the scanning electron micrographs were taken on a Philips FEI-XL30 scanning electron microscope.

Genomic DNA was isolated from dried specimens and the ITS1-5.8S-ITS4 segment from the ribosomal DNA (rDNA) was amplified with primer sets and ITS1 (5′-CTT GCG TTG ATT ACG-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) by polymerase chain reaction (PCR) techniques (White et al. 1990). A portion of the nuclear ribosomal large subunit (nrLSU) was amplified with the primers LR6 (5′-CGC CAG TTC TGC TTA CC-3′) and LROR (5′-ACC CGC TGA ACT TAA GC-3′) (Vilgalys & Hester1990). Amplified products were examined with agarose gel electrophoresis using a 2kb DNA marker. The amplified fragments were cloned into pCR2.1 TOPO (Invitrogen) to generate, the vector pCR2.1 TOPO containing the targeting fragment was transformed into its host *Escherichia coli* strain JM105 (López-García et al. 2003). The *E. coli* with the cloned fragments was sequenced by BGI Company and the data were deposited in GenBank. For the analysis of phylogenetic placement of the new species, the rDNA sequences of other related *Phylloporus* and *Xerocomus* taxa were downloaded from Genbank. Phylogenetic tree inferred from LSU rDNA sequences was constructed using maximum likelihood (ML) in Mega 5.05. Bootstrap analysis was implemented with 1000 replicates.

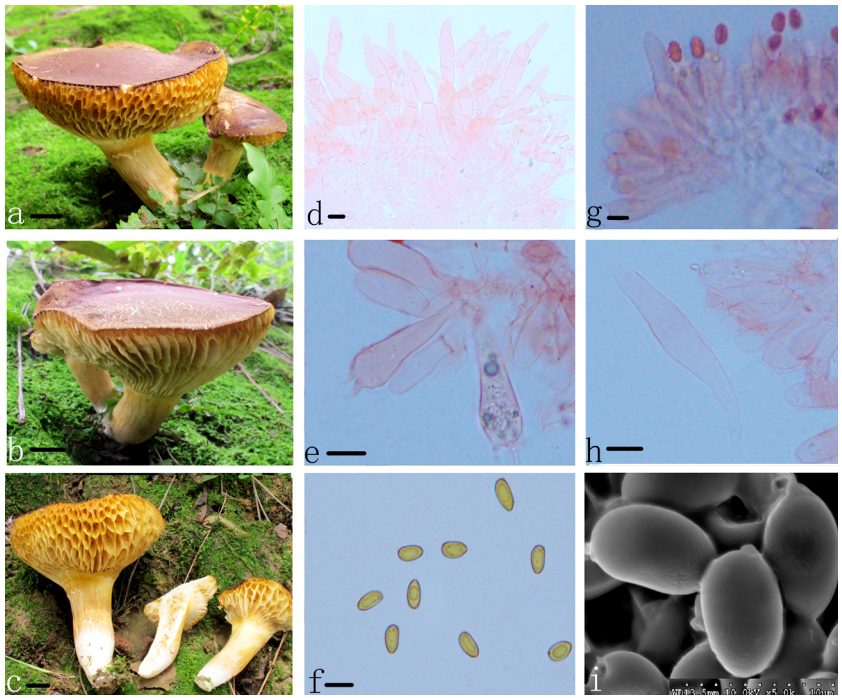

Fig. 1. *Xerocomus porophyllus* (GDGM 30303, holotype): a–c. basidiomes; d. pileipellis; e. basidia; f. basidiospores; g. hymenium with basidia, pleurocystidia and basidiospores; h. pleurocystidium; i. basidiospores under SEM. Scale bars: a–c = 1 cm; d–h = 10 μm.

Taxonomy

Xerocomus porophyllus T.H. Li, W.J. Yan & Ming Zhang, **sp. nov.** Figs. 1–2

MycoBank **MB 563347**

Differs from *Phylloporus cingulatus* by its larger basidiospores and non-cyanescent context on exposure; and from all species of *Xerocomus* s. str. by the largely lamellate hymenophore near the stipe.

Type: China, Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, 23°10.11′N 112°31.50′E, alt. 300 m a.s.l., 17 May 2012, Tai-Hui Li, Wen-Juan Yan & Hao Huang (**Holotype**, GDGM 30303; GenBank KC168089, KC561775).

Etymology: *porophyllus* refers to the partially poroid and partially lamellate hymenophore of the new species.

Pileus 50–80 mm broad, fleshy, convex, becoming plane to revolute with age, dry, even, subtomentose, matted subtomentose to slightly scurfy, often faintly and finely rimulose; grayish-ruby (12D5–6, 12E5). Flesh 5–20 mm thick at stipe, white to buff, sometimes with a very faint pinkish tint, unchanging

when injured, with mild odor and flavor. Hymenophore brownish-orange (5C4–6, 6C5–6), 2–5 mm deep, decurrent, lamellate near the stipe and strongly anastomosing, poroid or faveolate towards pileus margin, with 48–76 lamellae; pores 1.5–3 mm wide near pileus margin. Stipe cylindrical, 33–45 mm long, 5–15 mm wide at apex, attenuated at the base, dry, pale yellow to light yellow (4A3–4), solid when young, becoming hollow with age, with white basal mycelium; stipe context white (1A1), unchanging or with pale pinkish flush when exposed.

Basidiospores (5.5–)7.5–10.5(–12.8) × (4.0–)5.0–5.8(–7.0) μm [n = 30, Lm = 9.57 ± 1.17, Wm = 5.15 ± 0.86, Qm = 1.79 ± 1.41], ellipsoid, smooth under light microscope but nearly smooth to finely rugulose or weakly bumpy under SEM, yellow to olivaceous-yellow in KOH, inamyloid in Melzer's reagent. Basidia clavate, 28–34 × 8.0–11 μm, 2–4-spored, hyaline; sterigmata (2–) 3–5(–8) μm long. Pleurocystidia 42–68 × 9.0–12.5 μm, fusiform, thin-walled, hyaline. Caulocystidia and Cheilocystidia absent. Hymenophoral trama subparallel to nearly bilateral with hyphae of the lateral strata loosely arranged, hyaline, 4.5–19 μm broad. Pileipellis composed of tufts of erect hyphal elements 4–21 μm broad, terminal cells 11–55 × 4.5–20 μm, clavate, brownish-yellow to yellowish. Clamp connections absent in all tissues.

Ecology and Distribution — Solitary or clustered on soil under *Schima superba* Gardner & Champ. (*Theaceae*) in a mixed forest with *Pinus massoniana* Lamb. (*Pinaceae*) and other frondose trees. May to June. Known only from the type locality in southern China (Guangdong).

Comments — The new species is considered morphologically intermediate between *Phylloporus* and *Xerocomus* because of its dominantly lamellate hymenophore near the stipe, with the strongly anastomosing gills forming a faveolate or poroid pattern towards the pileus margin. The nearly smooth to finely rugulose spores observed under SEM are quite common in both *Phylloporus* and *Xerocomus subtomentosus* complex (Šutara 2008, Neves & Halling 2010, Zeng et al. 2012), which also implies that the two genera are closely related.

Many species of *Phylloporus* have a partially poroid hymenophore. The species most similar to *X. porophyllus* is *P. cingulatus* Corner with strongly anastomosing lamellae or poroid hymenophore, but differing in the slowly cyanescent context on exposure and the smaller spores (7.5–9 × 5–5.5 μm; Corner 1970). Among other species of *Phylloporus* with strongly anastomosing lamellae or poroid hymenophore are: *P. albocarnosus* Heinem., *P. ater* (Beeli) Heinem., *P. boletinoides* A.H. Sm. & Thiers, *P. carmineus* Heinem., *P. fibulatus* Singer et al., *P. ochraceobrunneus* Corner, *P. pumilus* M.A. Neves & Halling, *P. purpureus* var. *ambiguus* Heinem., and *P. testaceus* Heinem. & Gooss.-Font., which can all be easily distinguished from *X. porophyllus* by their pileus colors

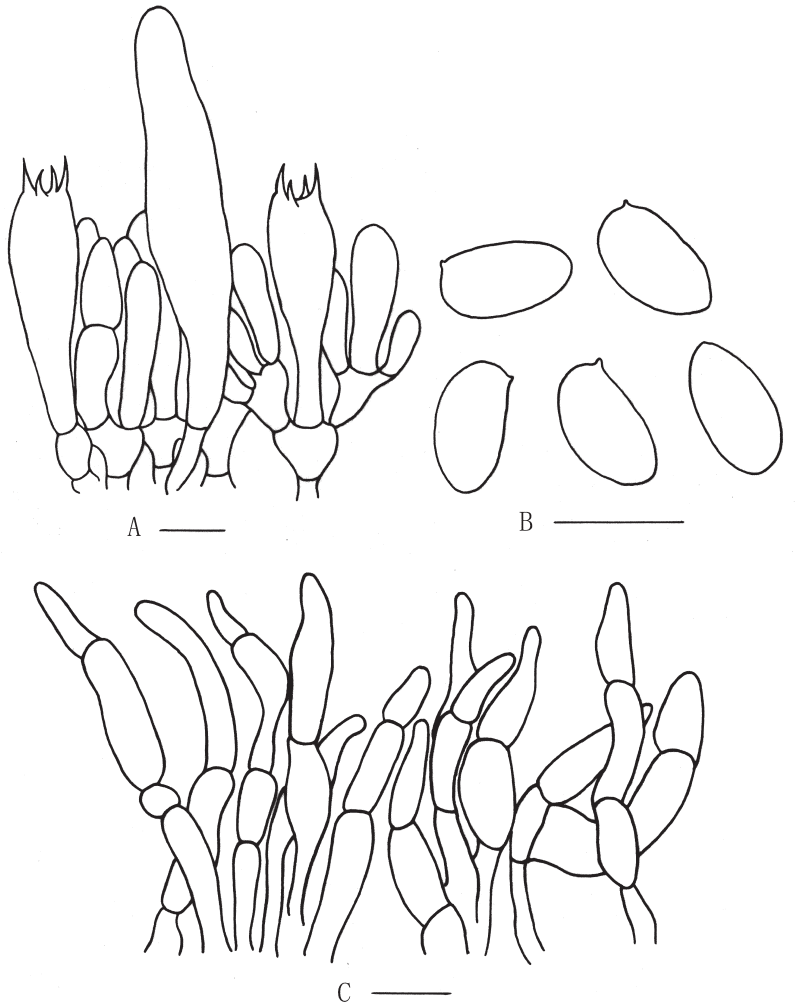

Fig. 2. *Xerocomus porophyllus* (GDGM 30303, holotype): A. basidiomes; B. basidiospores; C. pileipellis. Scale bars: A–B = 10 μm; C = 20 μm.

and sizes of basidiospores or basidiomes. *Phylloporus albocarnosus* has a white to straw-colored pileus and smaller spores (7–8 × 3–4 μm); *P. ater* is a blackish species; *P. boletinoides* shows a color variability ranging from cinnamon-brown or dark vinaceous-brown to cocoa-brown; *P. carmineus* is a carmine-red species; *P. fibulatus* has a much smaller (11–35 mm) and yellow to dull yellow pileus; *P. ochraceobrunneus* has fuscous-brown to brownish-yellow pileus and

Table 1. Sequence identity comparisons of *Xerocomus porophyllus* with species of *Xerocomus* and *Phylloporus*.

Species	Sequence	BLAST max score
ITS		
Xerocomus sp.	FJ197009.1	695 (88% query coverage) (85% max identity)
Xerocomus sp.	EU569235.1	695 (88% query coverage) (85% max identity)
Boletus dryophilus (≡ *Xerocomus dryophilus*)	AY185181.1	691
Boletus dryophilus (≡ *Xerocomus dryophilus*)	AY185183.1	686
Phylloporus sp.	JQ003620.1	368
Phylloporus sp.	JQ003650.1	359
Phylloporus rhodoxanthus	DQ533980.1	346
Phylloporus foliiporus	JQ003641.1	344
LSU		
Xerocomus fennicus	AF514820.1	1127 (100% query coverage) (94% max identity)
Xerocomus ripariellus	AF514818.1	1127 (100% query coverage) (94% max identity)
Phylloporus centroamericanus	JQ003663.1	1075
Phylloporus purpurellus	JQ003672.1	1016

larger spores (11–15 × 4–4.7 μm); *P. pumilus* has a very small (5–9 mm) cocoa brown or dark-brown pileus; *P. purpureus* var. *ambiguus* has a carmine-purple pileus and larger spores (9.3–12 × 4.2–5.3 μm); and *P. testaceus* has a rose-ochre to deep carmine-brown pileus and much larger spores (11.5–14.0 × 5.0–6.0 μm) (Heinemann 1951, 1955, Corner 1970, Singer et al. 1990, Neves & Halling 2010, Neves et al. 2012). Although some species of *Xerocomus*, such as *X. armeniacus* (Quél.) Quél. (≡ *Xerocomellus armeniacus* (Quél.) Šutara) and *X. chrysenteron* (Bull.) Quél. (≡ *Xerocomellus chrysenteron* (Bull.) Šutara), may have a subtomentose and finely rimulose pileus with similar color as *X. porophyllus*, their entirely poroid hymenophore is easily differentiated from the largely lamellate pattern of the latter.

The low max identities of the sequences demonstrate that the species compared are obviously different, and the higher max scores with the *Xerocomus* species also indicate that *X. porophyllus* has a closer genetic relationship with *Xerocomus* than with *Phylloporus* (Table 1). In a phylogenetic tree constructed with the LSU sequences of the new species, eight *Phylloporus* species and eight other *Xerocomus* species, with *Auricularia auricula-judae* (Bull.) Quél. as outgroup, *X. porophyllus* is grouped with the other *Xerocomus* species (Fig. 3).

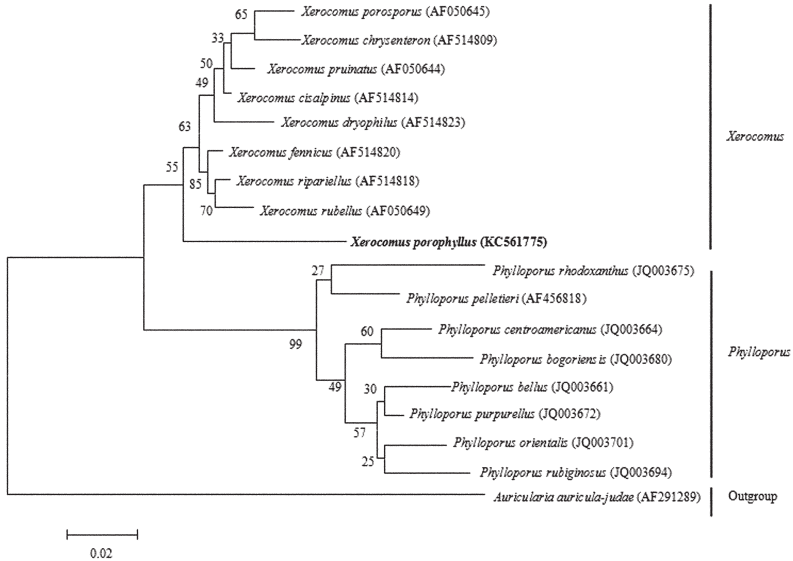

Fig.3. Phylogenetic placement of *Xerocomus porophyllus* inferred from LSU rDNA data. The bootstrap values (1000 replicates) are shown next to the branches. Taxa are labeled by their names and GenBank accession numbers.

Therefore, the novel entity is described as a new species of *Xerocomus* according to morphological and molecular analyses.

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